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CHAPTER 23

EFFECTS OF SEDIMENT SORPTION ON MICROBIAL DEGRADATION OF TOXIC SUBSTANCES*

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INTRODUCTION

Considerable attention has been focused recently on determining the environmental fate of phthalate esters (PAE) and other nonhalogenated industrial compounds. These ubiquitous compounds [1-4] have been associated with reproductive inhibition and bioaccumulation in *Daphnia magna*, a filter-feeding cladoceron [5,6], and rapid accumulation of phthalates has been observed in a variety of plants and animals within model ecosystems [7].

Microbial degradation has been shown to play a role in the fate of chlorpropham (CIPC) and di-n-butyl phthalate in the aquatic environment [8-13]. Little information exists, however, concerning kinetics of the microbial degradation of toxic substances entering water and sediment–water systems.

METHODS

Sediment Sampling

Samples of natural waters and sediments were collected from several sites within a 10-km radius of the laboratory in Athens, Georgia, and one from the

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Mississippi River below Memphis, Tennessee. Approximately 7 liters of water were collected from each site in two 4-liter, wide-mouth amber bottles. Sediments were collected at depths of 0–7 cm depth by an "Eckman dredge." Samples were transferred to wide-mouth amber jugs for transport to the laboratory.

Population Measurement

Microbial populations in water were measured by dilution-plate counting procedures. Populations were obtained for each sampling period by plating on TGE (Tryptone Glucose Extract Agar) and incubating for 72 hours. All counts were expressed as organisms per liter (org/l).

For sediment–water systems, the total adenosine triphosphate (ATP) in the system was used as a direct measurement of the mixed microbial population size by comparison to plate counts from water alone. The method employed was a slight modification of that reported by Bancroft et al. [14]. The sediment–water systems were extracted with 0.1 *N* $NaHCO_3$ (boiling), centrifuged at 15,000 rpm for 15 minutes followed by subsequent dilution with 0.1 *M* tris buffer. Total ATP was then measured through a bioluminescent reaction using luciferin–luciferase enzyme employing a SAI model/3000 integrating ATP photometer.

Residue Analysis

When compound disappearance could not be adequately measured by high-pressure liquid chromatography (HPLC), an appropriate aliquot of the reaction mixture was extracted with isooctane for analysis by gas chromatography (GC). Compounds requiring gas chromatographic analysis were determined by Ni^{63} electron capture using 3% SE-30 on gas chrom Q at 200°C and at a N_2 flow of 90 cm^3/min. HPLC analyses were performed by direct aqueous-phase injections of the reaction mixture, isocratic elution using 50:50 methanol:water on a Whatman ODS column at a flowrate of 2–2.5 ml/min, and detection by absorbance at a wavelength of 230 nm.

Kinetics Procedures

The kinetic procedure described below is representative of those used throughout the study. One-liter flasks containing natural water and/or Hickory Hill sediment plus water were incubated with test compound (chlorpropham or di-n-butyl phthalate). Equilibration concentrations of test compounds in the water remained below experimentally determined aqueous solubilities of 75±4.5 and 13±1.6 mg/l for CIPC and di-n-butyl phthalate, respectively. Sediment sorption was determined by equilibration of an aqueous solution of the compound with a known amount of adsorbent material. Sorbed compound was determined by difference and the resulting partition coefficient calculated. Based on the measured partition coefficients adjustments of the sorbent:water ratio were made such that from 15–90% of

the compound would be in the sorbed state. Flasks were incubated at 28°C under batch-shake culture conditions. Disappearance of test compounds was followed through a minimum of two half-lives, with the bacterial population being measured concurrently.

DISCUSSION

Laboratory and field studies [11,13] have indicated the reliability of a second-order rate expression to describe the microbial degradation of a pollutant in natural waters:

$$-\frac{d[S]}{dt} = k_2[S][B] \tag{1}$$

where $[S]$ = concentration of substrate or compound mg/l
$[B]$ = concentration of organisms, org/l
k_2 = second-order rate constant, liter/org/hr

Since the fate of such compounds are determined by both abiotic and biotic processes, we assumed that sediment sorption plays a significant role in determining the microbial availability of a toxic substance. Therefore, prediction of microbial degradation in natural systems requires the understanding of the effects of sediment sorption on the microbial process. The effect of sediments on the microbial degradation rate was treated by a modification of Equation 1, as shown below:

$$-\frac{d[S_T]}{dt} = \frac{k_2[S_T][B]}{1 + K_p[\rho]} \tag{2}$$

where $[S_T]$ = concentration of substrate or test compound, mg/l,
$[B]$ = concentration of bacteria, org/l,
K_p = measured partition coefficient for the test compound with respective sediment,
$[\rho]$ = sediment:water mass ratio, and
k_2 = second-order rate constant, liter/org/hr

The basic assumption is that sorption is rapid compared to microbial degradation and affects the kinetics only by decreasing the concentration of test compound.

To test the above equation, natural sediments with their overlying waters were sampled and incubated with test compounds. The results of an initial study of the microbial degradation of chlorpropham in Hickory Hill sediment are shown in Table I. These results were in direct contradiction with our initial hypothesis because the second-order rate constants were observed to increase with the sediment:water ratios. This was believed to be caused by errors in total population as determined by dilution plate counting of sediment–water samples. ATP analysis of sediment–water systems was therefore employed within all systems for population size determination.

Generally, populations exhibited a very fast increase within the first 24-48 hours of incubation. After this initial increase, populations were observed

Table I. Comparison of Second-Order Microbial Rate Constants with Increasing Sediment: Water Ratios

Compound	Sediment	Fraction Sorbed	k_2 (liter org^{-1} hr^{-1})
Chlorpropham (CIPC)	Mississippi River	0	$7.81 \pm 0.1 \times 10^{-13}$
		0.20	$2.82 \pm 0.4 \times 10^{-13}$
		0.90	$1.04 \pm 0.7 \times 10^{-11}$
	Hickory Hill	0	$6.51 \pm 1.2 \times 10^{-13}$
		0.20	$1.91 \pm 0.40 \times 10^{-13}$
		0.90	$6.03 \pm 1.2 \times 10^{-11}$

to remain high and relatively constant throughout the remainder of the incubation. Consequently, the disappearance of substrate (test compounds) was independent of population size, yet dependent on the concentration of available substrate. Further, a plot of the log of concentration vs time was linear with a slope of $k_{obs}[B]$, where $k_{obs} = k_2/(1 + K_p[\rho])$. An example of this treatment is shown in Figure 1. The pseudo-first-order rate constant, determined from a least squares analysis, was divided by total population (org/l)

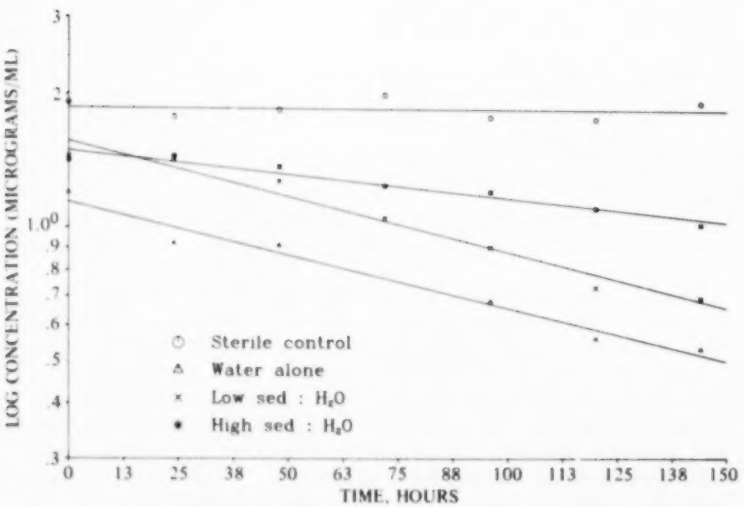

Figure 1. Pseudo-first-order plot of microbial degradation of di-n-butyl phthalate in natural water and sediment-water systems.

Table II. Comparison of Observed Second-Order Microbial Rate Constant (k_{obs}) vs Second-Order Rate Constants (k_2) Affected by Sediment Partitioning

Compound	Fraction Sorbed (Hickory Hill sediment)	k_{obs} (liter org^{-1} hr^{-1})	k_2 (liter org^{-1} hr^{-1})
Chlorpropham (CIPC)	0	$1.42\pm0.6 \times 10^{-13}$	---
	0.15	$4.15\pm0.7 \times 10^{-13}$	$3.45\pm0.2 \times 10^{-13}$
	0.90	$7.4 \pm1.2 \times 10^{-15}$	$2.29\pm0.6 \times 10^{-13}$
Di-n-Butyl Phthalate	0	$3.1 \pm0.8 \times 10^{-11}$	---
	0.15	$2.7 \pm1.5 \times 10^{-11}$	$3.47\pm1.7 \times 10^{-11}$
	0.90	$6.1 \pm0.5 \times 10^{-13}$	$2.60\pm0.21 \times 10^{-11}$

of the systems as determined by ATP to yield the observed second-order microbial degradation rate constant. Analysis of data in this fashion is consistent with the technique reported previously by Paris et al. [13] in the study of the degradation of several pollutants in natural waters.

Analyses of the data for the microbial degradation of chlorpropham and di-n-butyl phthalate at different sorbent:water ratios are presented in Table II. A reduction in the observed second-order microbial rate constant (k_{obs}) can be clearly seen as the sediment:water ratio is increased. The observed second-order rate constant (k_{obs}) corresponds to ($k_2/(1 + K_p[\rho])$ in Equation 2. A 100-fold increase in the sediment:water ratio reflected a 100-fold decrease in the second-order rate constant.

The second-order microbial degradation rate constants, k_2, were found to be comparable with the rate constants for the system without sediment. The second-order rate constant varied only by a factor of 2–4, depending on the compound studied. Rate constants for chlorpropham were within a factor of 2 for those reported by Paris et al. [13] for defined media and natural water studies. Other compounds and sediments studied have given similar results.

SUMMARY

The degradation of compounds at concentrations normally encountered in environmental samples can be adequately described by a second-order rate expression, which accounts for sorption in sediment:water systems. ATP is used as a measure of population size. It was shown that sorption of toxics to suspended sediments rendered the compound unavailable for degradation in the adsorbed state. Further testing of this kinetic expression is needed, however, for broader classes of compounds having wider ranges of sorptive properties.

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